National Aeronautics and Space Administration

Educational Product	
Educators & Students	Grades 9-12

FINAL - Number Pending

Educational Brief

NASA's Bioreactor: Growing Cells in a Microgravity Environment

How to Use This Brief

The purpose of this Education Brief is to provide teachers and students with inquiry-based, engaging, hands-on material that encourages conversation and higher order thinking, builds a deep knowledge, and makes connections to the world beyond the classroom. Activities in this brief meet National Education Standards and identify specific learning objectives.

Peruse the Standards and Objectives addressed in this publication while selecting particular items that will meet the needs of your classroom. To gain the needed content knowledge, study the sections prior to presentation of the lesson. Several online activities are also suggested with the readings for your own background knowledge or class time use.

Introduction

Biology is the study of life. When studying wild animals, biologists try to observe without disturbing the natural environment. The most natural environment ensures the most pure research. As in the study of wildlife, studying cells in their most natural environment gives scientists a better grasp of their true nature. For the most part, cells are studied and grown in flat, plastic petri dishes. Flat plastic is hardly a component of life and thus, not a natural environment for simulating the growth of cells in living organisms. Using spaceflight or a rotating wall vessel bioreactor, an environment can be created that more closely resembles the environment within the body. The rotating wall vessel bioreactor, or simply known at NASA as "the bioreactor" suspends cells in culture medium to allow three-dimensional freedom for growth.

Cell Science

Researchers have been culturing cells for more than a hundred years. Despite this century's many technological advances, techniques for cell culturing have not changed significantly. Today, cells are typically grown in petri dishes or in flasks, just

Activity One: Counting Breaths

Objective: To understand the importance of a pure environment for experimentation.

- Have two or more student volunteers come to the front of the class.
- Count the number of respirations of each student without him/her knowing using some distraction such as asking them to answer a question or taking the students' pulses. Count for fifteen (15) seconds and multiply by 4 to calculate a full minute of breaths.
- In a place only you can see, write down the number of breaths per minute for each student.
- State to the students and the rest of the class, "Now, I am going to count your respirations."
- Count them again. Write each student's name on the board and write their respiration rate underneath their names.
- Reveal that you took the students' respiration rates previously. Write down the first value next to each student's second value.

Discussion:

- Compare the numbers for each student.
- How is observation important?
- How can observation be wrong?
- What is student behavior like?
- What is it like with and without an adult in the room?
- What is the variable in an experiment such as this?
- What is an experiment you would like to conduct?

Focus Questions

- How are environments important to living things? Penguins in the Middle East, lizards in the Arctic Ocean, etc.
- What happens to cells in different environments?
- How do muscle cells react in an arm versus out in the sun?

Why do cells not differentiate in petri dishes?
Differentiation is simply the cell's ability to be different from a basic cell by specializing as a functional part of a larger tissue. Cells can differentiate to be muscle cells, skin cells, bone cells, kidney cells and actually much more distinct than that. Within a kidney, for example, there are many different types of specializing cells. Therefore, it may be easy to see that if a cell does not maintain its function and specialization when put in an artificial environment like a petri dish, it is very difficult to conduct research. However, some cells do differentiate in the rotating wall vessel bioreactor.

What is the limitation of cell culture in petri dishes?
Cells settle or sediment to the bottom of the dish.

What is sedimentation? Why is it bad for cell culture?
Sedimentation is the collection of cells on the bottom of a vessel due to gravity. This is bad for cell culture because cells are sedimented into layers meaning that the bottom-most cells have the complete weight of the cells above them pressing down on them. They also have less access to nutrients and oxygen than the cells above them.

What is one advantage to culturing cells in the true microgravity environment of space versus in rotating wall vessel bioreactors?
Cell clusters can get too large to hold in suspension on Earth in bioreactors and can only continue to stay suspended in space.

as they were a century ago. The cells are placed in these containers with a liquid **media**, a substance with nutrients the cells need to grow - and grow they do, in a flat layer on the bottom of the container. Cells grown ***in vitro***, meaning an artificial environment outside of the body, do not behave in the same way as cells grown ***in vivo***, or inside the body. Culturing cells in a petri dish is a less than ideal model of a natural environment for cells. Cells can grow along the flat, two-dimensional surface of the dish but as they grow upwards, on top of one another, their weight can terminate the cells beneath. *In vivo* cells grow **three-dimensionally** and form tissue that consists of cells that have changed their structure to perform a specific function in the body and other components, called matrix, that the specialized cells secrete. In vitro cells do not specialize, or **differentiate**. This poses obvious limits to researchers who want to understand mechanisms that govern cell behavior and tissue formation.

Beginnings Of Space Cell Biology

Though the limitations of standard culturing practices have been apparent for some time, solutions have been slow in coming. In the 1970s, a small group at NASA's Johnson Space Center (JSC) began to think about space as a possible answer. The group theorized that if cells could be grown without the influence of Earth's gravity, they would not settle, or sediment, to the bottom of the culturing container; rather, they would be suspended in the media and therefore might assemble and form tissue that more closely resembles tissue in the body. Although the goal was to attempt tissue growth on Space Shuttle missions, the JSC group soon turned their efforts to creating a culturing device that simulated some aspects of microgravity on the ground. The NASA team devised a system in which an upright cylindrical vessel - a bioreactor about the size of a soup can was rotated using an electric drill. With this setup, they were attempting to establish "suspension modality." But the team had not been able to achieve this state with the model system. Then one day, they decided to turn the rotating vessel on its side. That was the moment that everything went into suspension. Dr. Neal Pellis, Chief, Biological Systems Office, NASA JSC, states," That is how they discovered the rotating wall vessel bioreactor. From there, they realized that as long as they kept the cylinder completely filled with fluid, the cells should remain suspended." The NASA JSC team called the first devices the Slow-Turning Lateral Vessel (STLV) and the High Aspect Ratio Vessel (HARV), and they were ready for some serious testing.

Turning a Problem on its Side

Since the first testing of space cell culture, it has been understood that cells and tissue cultured in microgravity – the near weightless condition obtained in space – appear to express different genes than on Earth. A perpetual challenge for the experi-

mental study of these phenomena has been simulating the conditions of space so that complete laboratory studies can be done by numerous investigators on Earth. The simulated growth of mammalian cells in tissue culture needed to duplicate the tranquil conditions of orbital free-fall in a way that allowed for maintaining fresh media and oxygenation. To solve the problem, NASA in the 1980s developed the bioreactor, a can-like vessel equipped with a membrane for gas exchange and ports for media exchange and sampling. As the bioreactor turns, the cells continually fall through the medium yet never hit bottom. Under these tranquil conditions, the cells "self-assemble" to form clusters that sometimes grow and differentiate much as they would in the body. One limitation of the bioreactor is that, eventually, on Earth, the clusters become too large to be kept in a state of continuous free-fall causing serious damage to the cell clusters. This limits the amount of time an experiment can be run. It has been well established that a number of cell types grow in the bioreactor on Earth for extended periods in ways that resemble tissue-like behavior. For this reason, the bioreactor also provides cell culture researchers with a new tool for the study of three-dimensional (3-D) cell growth and differentiation. Bioreactors have been used aboard the Mir Space Station to grow larger cultures than even **terrestrial**-bioreactors can support. Several cancer types, including breast and colon cancer cells, have been studied in this manner. Research using the NASA Bioreactor is continued on Shuttle Missions.

The Basics of Rotating Wall Vessel Bioreactor Mechanics

The mechanics of the rotating wall vessel bioreactor are based upon **terminal velocity** and randomizing the **gravity** vector. Terminal velocity is simply the fastest velocity that an object can free-fall through a fluid. This is an equal balance of the force of gravity downward and resistance from the fluid upward.

As for gravity vectors, in physics, vectors are arrows that show the direction and magnitude of a quantity, such as the force of gravity. Earth's gravity vectors always point to the center of the earth (direction) with an acceleration of 9.8 meters/second/second or m/s^2 (magnitude). Although these vectors will always point "down," an object that is tumbling, changing its **orientation** randomly is affected by gravity in all directions randomly.

Taking a cylinder filled with cells and culture medium, then rotating it on its side makes for an environment similar to spaceflight. Cells move with the liquid medium and the medium moves almost as one single body, somewhat like a solid. Cells are held suspended in the medium at a balanced speed between too fast causing centrifuging and too slow allowing sedimentation. The cells receive oxygen from an integrated membrane, which is also able to remove carbon dioxide by partial pressure. Cells float and tumble similar to experiments done in spaceflight. An environment similar to spaceflight is similar to an environment inside the body.

Lead-in Discussion:

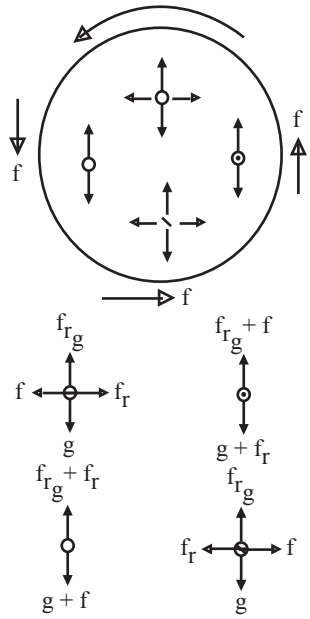
If gravity on earth always points in the same direction, how can the effect of gravity be randomized?

Visualizing the answer:

If you were as tiny as a cell and tumbling in a bioreactor, sometimes the earth would be below your feet. Sometimes the earth would be above your head, if you were up-side-down relative to the earth. Whatever direction you have to look to see the earth, that is the direction the gravity vector is pointing.

Have students explain the effects of rotating bioreactors in their own words.

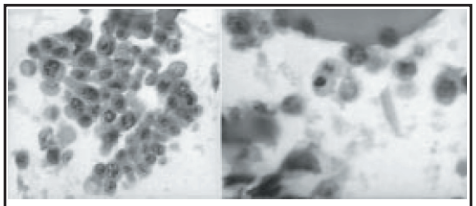
Draw a force vector diagram of a cell rotating in a lateral rotating wall vessel bioreactor. Consider 2 perspectives down the axis of the vessel. Show the cell at the top, downside, bottom and upside.



Where:

- g = force of gravity acting on cell
- f = force of rotating fluid against cell
- f_r = force of fluid resistance against cell
- f_{r_g} = force of fluid resistance opposing gravity

What are the organizational levels of living things? cells, tissue, organs, systems, organism



Microgravity **Ground**

These photos compare the results of colon carcinoma cells grown in a NASA Bioreactor flown on the STS-70 Space Shuttle in 1995 ground control experiments. The cells grown in microgravity (left) have aggregated to form masses that are larger and more similar to tissue found in the body than the cells cultured on the ground (right). The principal investigator is J.M. Jessup of Georgetown University, Washington D.C. Credit: NASA.

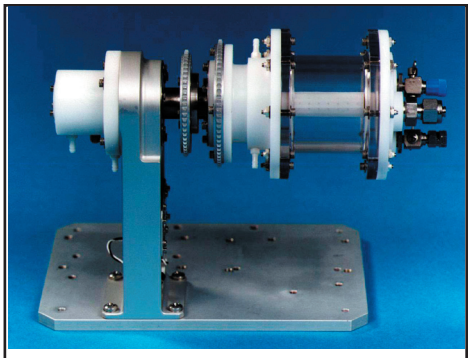
While on orbit the cells do not need to be suspended to float, the rotation of the bioreactor helps in other ways. One of the main advantages is known as "mass transport." This simply means that as the cells move through the culture medium, they will run into nutrients and oxygen. This is advantageous because in space, movement of molecules through a stationary medium occurs simply by diffusion. Rotation affords cells a higher probability of engaging necessary nutrients for their growth and increases their ability to remove wastes.

An Early Believer

In 1987, J. Milburn Jessup was working at the University of Texas M. D. Anderson Cancer Center with his mentor, I. J. Fidler. Fidler's main interest was in understanding **metastasis**, or how **cancer** cells spread from a primary to a secondary site in the body. Fidler wondered whether there was something about the three-dimensional structure of a host tissue that made it susceptible to colonization by **malignant** cells. "We were thinking along the lines of trying to get some sort of culture system that would mimic some aspects of this three-dimensional growth," remembers Jessup. Dr. Neal Pellis, then also working at the University of Texas Health Science Center, Houston, and a friend of Jessup's, recommended that he go to the Johnson Space Center (JSC) where an old colleague, Thomas Goodwin, was working with the group that was trying to devise a new culture system. The device Jessup saw when he went to JSC was far beyond the original cylinder driven by an electric drill design. Jessup remembers being impressed by the NASA cell culturing system: "They had long-duration motors and parts that seemed to me to indicate that the engineering aspects of this were really very well thought out. This bioreactor prototype was likely to be rugged and durable. At that point, they were looking for cells to put into it." Jessup was sure this system held promise. "I wasn't skeptical," Jessup recalls. "I was more drawn by how dedicated this group was to working hard to develop a product. They needed help in terms of resources and supplies, which we could offer, to make this bioreactor functional. I didn't have any hesitation that this would in fact be a useful culture system. I thought it was perfectly logical."

Jessup's confidence turns out to have been well-placed. The JSC group had tried culturing hamster kidney cells in the type of bioreactor called a Slow Turning Lateral Vessel (STLV), but the results had not been what the group had hoped for. Jessup first provided JSC with a simple human colorectal **carcinoma** cell line. Then, when that met with success, the group wanted to attempt a cancer cell/normal cell interaction – a **coculture**. Jessup described the result: "Fairly large tissue **aggregates** grew, and these had the ability to really recapitulate the morphology or appearance of what occurs *in vivo* in mice." Three-dimensional tissue masses, resembling a cancer tumor, had grown in the STLV. "Some years later," said Jessup, "those results were published. They demonstrated that the vessel was very good for these kinds of cocultures. We demonstrated a synergy not evident in other culture systems."

Work began on preparing a culturing system for a shuttle flight. Although the bioreactor would be in a microgravity environment aboard the Space Shuttle, and the cells would therefore be in suspension without vessel rotation, the same system developed for the ground was modified for use in space. The cylinder that is the rotating bioreactor is just a part of a larger system designed for keeping cells alive by providing all the resources they would have in a body. Pellis said that JSC had developed an integrated system: "It has a reactor – the culture vessel itself – but in addition, it has its own lung, its own heart, its own food supply, and its own waste management." Adapting that system for space hardware rather than starting from scratch made the most sense.



The Slow Turning Lateral Vessel (STLV)

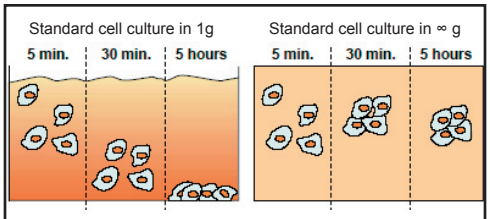
Jessup was the first investigator to use the space hardware, although he explains that his experiment was primarily a test of the space system. JSC designed the experiment, and he provided the cells and the analysis. Once again, Jessup was providing resources that JSC needed in its quest to make their rotating wall bioreactor a functional, useful device. After several spaceflight trials, JSC's Bioreactor Demonstration System flew onboard **Space Transportation System** (STS)-70 in July 1995, with cells provided by Jessup. The experiment was not only an engineering success but also a scientific one. Jessup's sample of colon carcinoma cells aggregated to form masses 10 mm in diameter. These masses were 30 times the volume of those grown in the control experiment on the ground. The experiment was repeated in August 1997 on STS-85, and mature, differentiated tissue samples were grown again, confirming the previous results - microgravity was an environment beneficial to cell culture and tissue growth.

Interest in the rotating bioreactor is developing, for the most part, on two sides of a fence: One side is applications, meaning building tissue, whether it is tissue for research or for **transplantation**. The other side is study of those properties of cells that change because the cells are in free-fall. In the next five to ten years, the rotating bioreactor will begin to routinely produce tissue for research and transplantation. The tissue produced to date in the rotating wall vessel has already offered unique research opportunities. "This is the first time," says Pellis, who now leads the NASA Exploration Cell Science Program at JSC, "that we have a look at the dynamics of a three-dimensional arrangement in a cultured setting. For instance, we can grow a human colonic polyp from individual cells. Observing that particular three-dimensional dynamic is an investigation of cancer that can lead to the development of therapeutic treatments. That is not something from 'Ripley's Believe It or Not.' That is going to happen." The National Institutes of Health (NIH) also believes it is going to happen. Sixteen research projects involving tissue culturing in rotating wall bioreactors are currently under way at the joint NASA/NIH Center for Three-Dimensional **Tissue Culture** at the Institute of Child Health and Human Development in Bethesda, Maryland. The two agencies joined to form this center in 1994 under an agreement that the NIH would provide the lab and NASA would provide rotating bioreactors and other support. The combination of NASA technology and NIH expertise has already resulted in the successful culture of several **infectious** agents that are difficult to grow and control in a conventional culture setting. Pellis points to the growth of Cyclospora, a **parasite** that lives in berries and causes extreme gastrointestinal distress when eaten, as an example of the project's success: "No one has been able to grow Cyclospora in culture until recently, when researchers at the joint center took a new approach and cultured the organism with cells from the small bowel." The tissue samples grown in the rotating bioreactor at the Center are being used to design therapeutic drugs or **antibodies**, "or alternatively," said Pellis, "for designing a strain of the organism from which a vaccine could be produced." Cyclospora is not a big threat in America, but worldwide it is responsible for a significant percentage of infant deaths from dehydration. Researchers at the joint Center have also had success culturing the human immunodeficiency virus (HIV-1). HIV has been propagated before without the rotating bioreactor, but at the joint center, the NASA technology has made possible the propagation of the virus in human lymphoid tissue. Those samples are giving scientists an opportunity to observe the virus in full dynamic process, which should provide a new perspective on the **disease** and on possible treatments.

A Novel Look

The other side of the fence in bioreactor research is using the culture technique to gain what Pellis calls "a novel look at the cell." Pellis notes that while using the bioreactor to engineer better tissue samples, several researchers have observed that cells adopt some interesting adaptive modes while free-falling in the rotating bioreactor on the ground and in orbit. Pellis believes that by watching the

During the STS-90 shuttle flight in April 1998, Hammond's human renal tubular cells formed large tissue aggregates (visible as white masses in the lower left corner of the sample above). Hammond was able to use the samples to identify key genes in the control of differentiation.



Cell constructs grown in a rotating bioreactor on Earth (left) eventually become too large to stay suspended in the nutrient media. In the microgravity of orbit, the cells stay suspended. Rotation then is needed for gentle stirring to replenish the media around the cells.

reaction of the cells to the new environment of suspension or microgravity, scientists will discover more about the mechanisms that control the cells' behavior. "Besides being able to grow tissues," said Pellis, "we now have a new and fascinating way to see inside the cell."

Seeking a Solution

Timothy Hammond is an example of a researcher who started out using the bioreactor to engineer tissue and ended up using it to find the mechanisms within the cells that control differentiation. Hammond and his team at the Tulane Environmental Astrobiology Center, which is jointly sponsored by Tulane University, Xavier University, and the VA Medical Center in New Orleans, were studying **protein** receptors that bind common toxins in the proximal tubule, a microscopic tube in the kidney. Hammond explained that the kidney often is damaged by drugs and toxins contained in strong antibiotics. "We were interested in the proteins that get bound in the kidney by these toxins. We wanted to culture the cells that make these proteins, to develop protective agents," said Hammond. "But the problem is that there is no cell culture line that expresses the relevant proteins. If you take a kidney cell and put it into a cell culture, a day later it no longer has any of its special features. So we had a lot of interest in finding a cell culture method that would keep the special features of tissues intact."

Hammond tried over 400 different cell types and cell culture techniques searching for a way to retain the special features of the differentiated cells of the tubule, such as microvilli, hair-like structures found in some tissues. He met with no success. Then he read about results of culture experiments in the rotating bioreactor, and he immediately contacted NASA. "We tried the rotating wall vessel," remembered Hammond, "and to our shock, surprise, and delight, the tissue was beautiful. All the hair, the microvilli, grew on the cells, and they expressed all the specialized proteins we needed. The results were very dramatic."

Though these results were striking, Hammond thought that culturing the cells in space might produce even more spectacular samples because in orbit, the tissue masses would be less limited by size. On Earth, when the cells aggregate into three-dimensional masses in the rotating bioreactor, they eventually reach a size at which they are too heavy to be suspended by the rotating action of the vessel. If the rotation is increased to keep the aggregates suspended, they are thrown against the vessel wall, which damages the tissue. "If we were truly going to understand how different cells grow together to form a tissue with all its medical implications," said Hammond, "we had to find some way to get out of the limits caused by gravity. That is why we wanted to fly the **renal** tubular cells."

Hammond's first opportunity to conduct an experiment in microgravity was during the sixth Mir research increment, from September 1997 to January 1998. Hammond chose to grow rat renal tubular cells in NASA's Biotechnology Specimen Temperature Controller (BSTC), a cell **incubator**, onboard the Russian space station Mir. He chose rat kidney cells for his sample because he needed cells that would grow and differentiate over the entire four months to help verify the function of the hardware. Hammond reported that on Mir, the tissue aggregates "grew beautifully" under the care of astronaut David Wolf, one of the rotating wall bioreactor's three inventors. "We got gorgeous cell aggregates, bigger than the aggregates grown in the control experiment on the ground," said Hammond. "And we saw

the proteins that we were interested in, the tubular toxin protein receptors, expressed in flight."

Hammond was pleased with these results, but he wanted to know what mechanism in the cells was causing differentiation and **expression** of the desired proteins in microgravity. **Genes** control these functions, but identifying which genes are doing the controlling out of the millions present in a cell is close to impossible. Hammond reasoned that a comparison of the genes that are active in the cells during culturing in spaceflight to those that are active in culture on the ground might help in pinpointing the specific genes responsible for differentiation. In early 1998, Hammond cultured a sample of human renal tubular cells in the bioreactor on STS-90 for six days. Hammond reports that by comparing the activity of 10,000 genes in the flight and ground cultures, several of the control genes for differentiation and three-dimensional tissue formation were identified. Hammond eventually wants to use these findings to make kidney implants for hormonal therapy. "With the knowledge of the control genes," says Hammond, "we could control the proteins produced by tissue in the rotating vessel by genetic manipulation so we can give the patient a better, longer-lasting implant. Our experiment is a very exciting piece of basic science, but it does have clinical correlates." Hammond is certain that the rotating wall vessel will bring such success to many other researchers in the future. "I believe that NASA's biotechnology program is going to revolutionize the whole field of cell biology," says Hammond. "In fact, it already has."

Astronaut David Wolf makes notes about Hammond's sample of rat renal tubular cells (above his head) onboard Russian Space Station Mir. Wolf is one of the coinventors of NASA's rotating wall bioreactors.

For more information, go to www.nasa.gov and search the title "Culturing a Future."

A Keyhole to the Future

While the rotating bioreactor is providing researchers with a new way to see inside a cell, it is also expected to contribute to our efforts to look out into our solar system and beyond. Jessup and Pellis share the view that research conducted in the rotating bioreactor will be a prerequisite for space travel and colonization. Jessup sees the primary role of the cell culturing program as helping to ensure astronaut health. The program can do this, he says, through research that "provides the underpinnings for many of the health disorders that occur in space, such as anemia, bone matrix loss, and kidney stone formation." Jessup points to investigators already using the bioreactor to solve these problems. Among them are Dr. Neal Pellis, who has done work examining the behavior of immune cells in microgravity that may lend insight into the changes astronauts experience in their immune systems during spaceflight, and Lisa Freed and Gordana Vunjak-Novakovic, researchers at the Massachusetts Institute of Technology, who believe that results from their experiment to grow **cartilage** on Mir might provide clues for understanding why astronauts experience a weakening of muscle and bone while in space. (See "Women of Science").

Bioreactor Potential

Jessup also sees a continued role for the rotating bioreactor once astronauts are en route to new planetary destinations. The bioreactor can provide a means for culturing red blood cells or skin in the event of astronaut trauma. It can also be used to culture unicellular organisms like blue-green algae as a supplemental food source or a means of replenishing the air supply for the spacecraft or for a planetary colony. "Because such organisms are biologically renewable," Jessup says, "they may be cheaper in the long run than chemical agents that could be used to create air and easier to transport." Pellis adds that there is potential for using the findings from bioreactor research to send cells into space as

Lisa Freed (center), Gordana Vunjak-Novakovic (right), and their graduate student Bojana Obradovic (left) with NASA's rotating bioreactor containing engineered cartilage. Photo credit: Donna Coveney, MIT

Activity Two:
Objective: To discover and share biographies of the women involved in biology or biotechnology research and their contributions.
By using websites such as http://microgravity.nasa.gov/WOMEN/ or http://quest.nasa.gov/women/WON.html or others, students should write a report and plan a presentation preferably using PowerPoint or their own drawings on poster board.

exploratory probes. Cell cultures could be designed to respond to environmental conditions of other planetary bodies in such a way that scientists could judge whether an environment is suitable for life. "Using these probes," says Pellis, "we could determine if the atmosphere is supportive of cells, if there is water, or if the environment is amenable to **propagation**."

Though Jessup is enthusiastic about the contributions the bioreactor will make toward engineering tissue on Earth and toward the study of novel aspects of cell biology, it is the program's role in future space exploration that he finds most compelling: "In the next millennium, we will move off the Earth," says Jessup, "and quite frankly, I think that this bioreactor technology is the primitive forerunner of the technology that will enable us to do that. The bioreactor represents a keyhole to the future."

Application of Space Cell Culture

Women of Science

In their 12 years of working together, Lisa Freed and Gordana Vunjak-Novakovic have shared some big moments. Witnessing the spectacular night launch of the Space Shuttle that carried their tissue culture experiments to the Russian Space Station Mir, in September 1996 was one; finding out, four months later, that the cartilage cells had survived their stay in microgravity was another. The researchers met in 1986, when Freed was a graduate student and Vunjak-Novakovic was a Fulbright scholar at the Massachusetts Institute of Technology (MIT). The two worked together on a bioreactor to detoxify blood. Although it was not a tissue growth project, the eventual focus of their research together, it demonstrated their potential as partners. Says Vunjak-Novakovic, "We discovered that we worked very nicely together, and that has continued over the years." Freed points out that they each bring different strengths and backgrounds to their work: "I was trained in medicine and biotechnology, and Gordana in chemical engineering. Gordana is stronger in math, and I have a little more experience in the medical side of things."

Their NASA research began when Freed became interested in tissue engineering as a postdoc at MIT. She was using conventional bioreactors to culture cartilage and heard of NASA's rotating bioreactor. "It looked like a very promising culture system," remembered Freed, "because the cells could be grown in free suspension without a lot of potentially damaging **shear forces**." The rotating bioreactor keeps the cells suspended without resorting to the use of stirrers that cause shear and damage tissue. Freed submitted a proposal to NASA and was awarded a grant in 1992. With Vunjak-Novakovic as her co-investigator and two rotating bioreactors in their lab at MIT, Freed embarked on ground-based studies to grow cartilage. Vunjak-Novakovic remembers that they were surprised at first "by how good the tissues were that we could grow in the bioreactors," but she notes that it is not unusual for research to start with a surprise: "There is a learning curve in this kind of work. You design an experiment and really discover something, and then you try to take advantage of that discovery and design another experiment to follow up on it."

In 1994, the team was selected by NASA for a spaceflight opportunity. Cartilage is a model cell system, and it was hoped that cartilage tissue grown in space might provide clues to understanding and perhaps preventing or treating the weakening of **musculoskeletal** tissue that astronauts experience

during spaceflight. “The information that you learn from experimenting with cartilage cells,” explained Freed, “can be extended to other cells, like muscle or bone cells.” In addition, cartilage requires less oxygen and fewer nutrients to survive than other tissues, which made cartilage perfect for Mir, where resources were limited. The survival of the cartilage cells over four months in microgravity demonstrated that long-term spaceflight studies are feasible. The space-grown tissue, when compared to the control experiment on Earth, also revealed some interesting differences. The cells from both the space and control experiments were healthy, but the tissue that grew in microgravity was mechanically weaker and smaller than the tissue that formed in the ground experiment. Freed explained that the space-grown tissue contained less of a key component, **glycosaminoglycan**, that the cells secrete. Vunjak-Novakovic said that this clue may help them to understand not only the weakening of tissue experienced by astronauts but also similar diseases experienced by people on Earth. Freed reported that they have already received calls to their lab from people who suffer from cartilage problems. “They are waiting for what we are doing in the lab to be ready for them,” she said. “It is always hard to explain that research goes very slowly and that we are working as hard and as fast as we can.” Freed and Vunjak-Novakovic share the dream that tissue may one day be engineered for replacement of damaged or congenitally defective tissue. Their research is bringing the realization of that big moment ever closer.

Activity Three:

Objective: To discover factors that impact gravity (g) and to calculate the value of gravity.

Gravitational Pull
http://media.nasaexplores.com/lessons/01-002/9-12_2.pdf

Microgravity Overview

Many people are quite familiar with the images of astronauts floating in space or candy and food hanging in midair of the Space Shuttle or International Space Station (ISS). These examples of floating are actually examples of falling, orbital free-falling. Often this environment is referred to as “microgravity” or weightlessness. The Greek word *micro* means “small” and is used in science as a prefix meaning one-millionth or 10^{-6}. Free-fall experiments are conducted on Earth, too, in several ways such as drop towers, parabolic flight, sounding rockets, and bioreactors. Each of these methods utilizes gravity by raising subjects up, increasing their potential energy, and then dropping them. As the objects fall, they are allowed complete three-dimensional freedom of movement or what looks like floating.

Fun Fact:
If you were to stand atop a mountain at the height of Low Earth Orbit (LEO), you would still weigh between 85% and 95% of your weight at sea level.

- Drop towers release experiments, usually down a shaft, to allow 2-10 seconds of free-fall.
- In parabolic flight, large aircraft such as a KC-135 or DC-9 loop up and down, similar to a roller coaster. As the aircraft reaches the top of a “hill,” the pilots cut back on the engines allowing the plane to begin a dive. When the plane is falling, whatever is inside is falling, too, also looking like floating for 20-25 seconds.
- Sounding rockets launch their experiments, or “payloads,” to heights of up to 800 miles and then allow them to fall back to earth for free-fall of 4-12 minutes.
- Bioreactors rotate their contents in a fluid to maintain a suspension. Cells can culture in rotating wall vessel bioreactors for days and weeks at a time with constant and consistent effects of microgravity.
- Bed rest, while not being a free-fall method, is an analog of microgravity for studying the effects of the lack of loading. Astronauts’ muscles and bones get weaker from the lack of use on orbit. Similarly, test subjects rest in bed for months at a time to achieve some of the same muscle and bone loss.

Activity Four:

Objective: To demonstrate that free fall eliminates the local effects of gravity.

Free Fall Demonstrator
http://science.msfc.nasa.gov/msl1/ground_lab/exp2.htm

When viewing a Space Shuttle launch, you may have noticed the enormous force used to accelerate the Space Shuttle in an arching path. The Shuttle must accelerate until it reaches orbital velocity. Orbital velocity is maintained at approximately 17,500 miles per hour. For missions to the Moon, Mars, and beyond, vehicles need to reach escape velocity. Escape velocity (http://media.nasaexplores.com/lessons/01-049/9-12_1.pdf.) is the speed a body must attain to break free of the gravitational hold of the larger body. Escape velocity depends on the mass of the larger body and the distance of the smaller body from the larger body's center.

You can see an animation explaining a shuttle launch at this web site http://kids.msfc.nasa.gov/rockets/shuttle/launch.asp. Then, search for a Space Shuttle launch video at http://mediaarchive.ksc.nasa.gov.

While all of these microgravity analogs have their benefits, orbit allows more long-duration, more pure microgravity experimentation. Traveling at approximately 17,500 miles per hour, the Space Shuttle and International Space Station (ISS) balance the force of gravity pulling them down with a force that is parallel to the earth's surface. This balance of forces allows these spacecraft to free-fall almost indefinitely. Mistakenly, many people think that astronauts escape gravity by going so high in orbit. It is not a matter of the distance from earth that affords microgravity to the Shuttle and ISS. Nor do astronauts escape gravity. It is the balance between the pull of gravity and the velocity of the spacecraft that keep them in orbit. If astronauts escaped gravity, they would be on their way to the outer reaches of the solar system!

This effect of orbit is used in many different science arenas including: material science, combustion science, fluid physics, fundamental physics, and biotechnology. Specifically in biotechnology, cell science has made tremendous strides employing the environment of orbital spaceflight. Spaceflight allows cells to float within the culture medium instead of sinking to the bottom as they do on earth, known as "unit gravity" or "one gravity" (1g). On orbit, gravity does not hold cells down to a surface nor does gravity compress the weight of cells growing on top of others upon the cells below. In spaceflight, what is "down"? There is no "top." Nothing is "below." Cells have complete freedom to grow in all three dimensions.

Classroom Bioreactor

The Classroom Bioreactor (CB) is built from parts that are easily obtained and assembled. It uses a toy rock tumbler and a polypropylene specimen jar. The easy to adapt plastic tumbler supports the specimen jar and is designed to be built and used in a classroom setting. The CB will primarily be used to examine the effect of microgravity on **germination** of seeds, but also has the potential to be autoclaved and used **aseptically** for other tissue culture experiments similar to the NASA-designed ground-based rotating wall vessel bioreactors and the flight-based Bioreactor Demonstration System. This is a very user-friendly, versatile system and can be easily implemented into school settings unlike commercially available sophisticated Rotating Wall Vessel Bioreactors (STLV and HARV). Also, this bioreactor generates the analog simulated microgravity environment and has the potential to mimic the *in vivo* host **microenvironment**. The Classroom Bioreactor offers easy handling of any experiment to be performed with any biological system.

Classroom Bioreactor Construction Directions

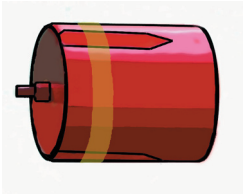
Classroom Model Bioreactor
Parts List and Instructions

CAUTION: This Classroom Model Bioreactor employs an electrically operated toy and is not recommended for use with children under 10 years of age. As with all electrical products, precautions should be observed during handling and use to prevent electric shock. Do not immerse in water. Wipe clean with a damp cloth. Keep hands, fingers, and other body parts away from saw blade and scissor blades. Make sure to read silicone handling and ventilation safety precautions.

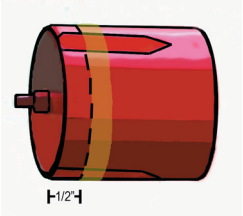
1 Toy Rock Tumbler ($20-$45), found at hobby and craft stores. Be careful not to accidentally purchase the "Refill Kit." The boxes look almost identical except for a small label that says "Refill Kit." (recommended Rolling StonesTM from Natural Sciences Industries, Ltd. / Smithsonian)
1 Nalgene Polypropylene screwcap jar (125ml) (i.e., Fisher Scientific PN#02-890-15C – 36pk/$60.00) https://www1.fishersci.com/
1 Caulk gun (for 10 oz. cartridges)
1 Silicone II Cartridge (10.1 fl. oz. / 299 mL) found with caulk cartridges
1 Miter box and hand saw
1 Insert template sheet found at http://slsd.jsc.nasa.gov/bso/resources/?viewFile=classroomActivities
1 Pair of scissors
1 Roll of cellophane or masking tape
1 grease pencil
2 wide rubber bands
Petroleum Jelly

1. Remove lid of tumbler barrel and set it aside for use in step 8.

2. Make a ring with a piece of tape around the outside of the tumbler barrel with the edge of the tape approximately ½ inch (1 cm) away from the bottom of the barrel. (A little more than ½ inch is better than a little less.)

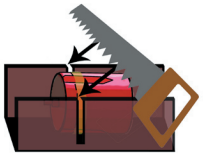


3. Use a grease pencil to make a line around the outside of the barrel along the tape ½ inch from the bottom of the barrel.



4. Remove the tape from the barrel.

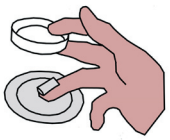
5. Place the barrel in the miter box and using the saw, carefully cut off the bottom along the grease pencil line.



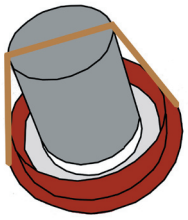
6. Using a pair of scissors, carefully cut out the insert templates.

7. Tape the templates into their appropriate container with the printed side facing out (inside of the lid and bottom of the barrel).

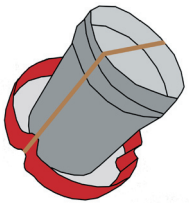
8. Place a small loop of tape, sticky side out, on the top of the cap of the polypropylene screw cap jar."



9. Adhere the cap to the place indicated on the barrel lid template. (TIP: use an additional jar in the cap to provide more support with a rubber band).

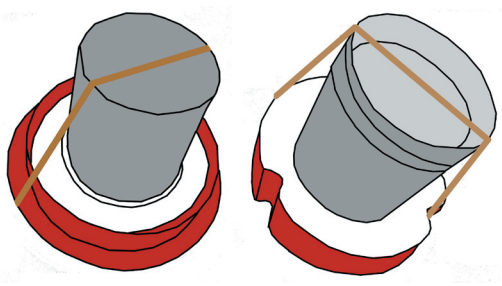


10. As in steps 6 and 7, tape the jar with a loop of tape to the place indicated on the barrel bottom template.

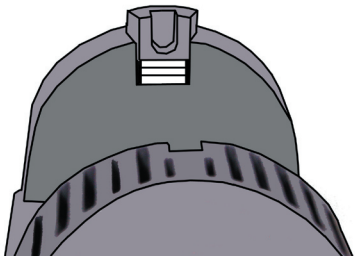


11. Use a rubber band to add additional support to the jar.

12. Follow cartridge instructions on how to use a caulking gun and fill the space in between the jar/cap and bottom/lid with silicone (TIP: Make 2-4 thin, single passes around the jar or cap to fill in the space. Trying to fill slowly with one single pass increases curing time.) Make sure to read handling and ventilation safety precautions.



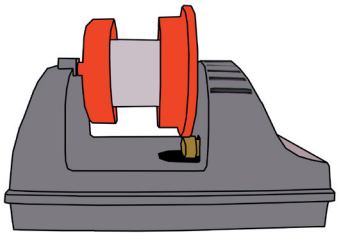
13. Prop the adjustable axle wear plate (see Rock Tumbler diagram) about ¼" (0.7 cm) with a piece of Styrofoam or folded paper to make the barrel level.



14. Once the silicone has hardened (24 hours), peel the gaskets free from the tumbler, jar, and template. The templates may be thrown away. The gaskets are exchangeable with other Classroom Bioreactors.

15. Apply a small amount of petroleum jelly, (enough to "grease"), the axles and axle wear plate. This will help decrease the wear on the axles.

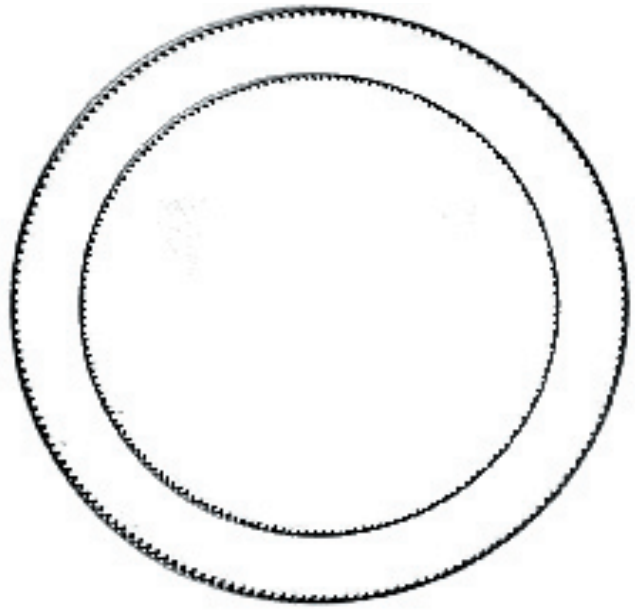
16. Place the tumbler-jar and gaskets assembly onto the Rock Tumbler body and turn on the Rock Tumbler to make sure the construction rotates freely and is level.



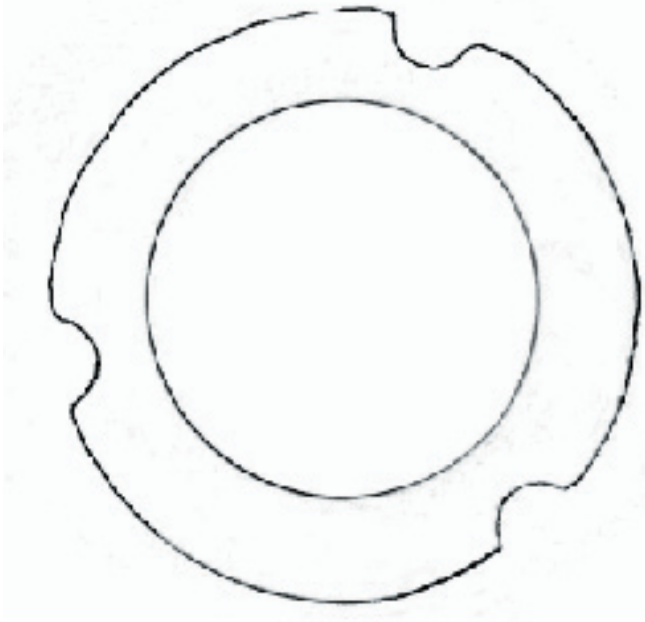
Now, you have a Classroom Model Bioreactor!

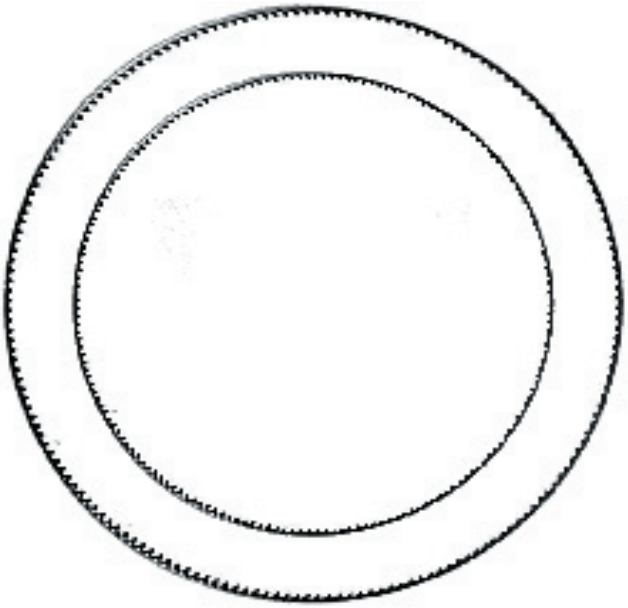
Classroom Model Bioreactor Gasket Templates

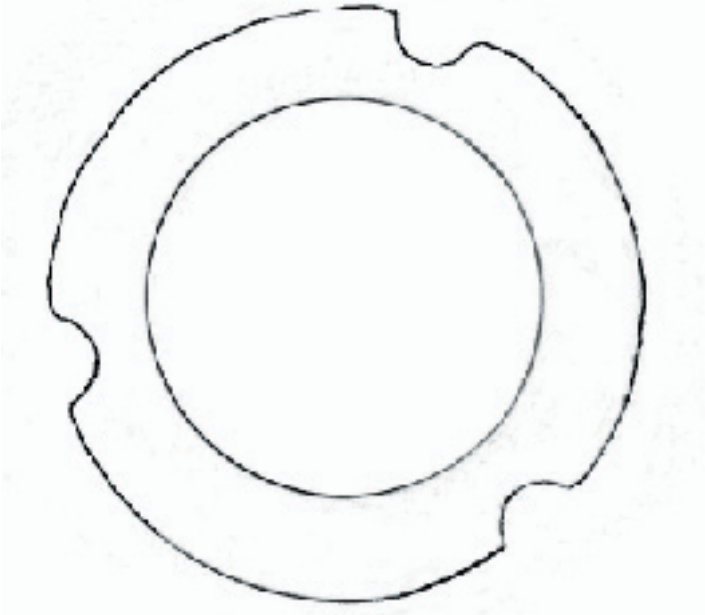
Tumbler Lid / Specimen Jar Lid Template



Tumbler Barrel / Specimen Jar Template







Classroom Bioreactor Lesson: Seeds in Motion

Seed Growth in a Classroom Model Bioreactor

Objective: To investigate and compare the growth of seeds in 1g under stationary conditions and simulated microgravity in a rotating wall vessel.

Activity Time: 10 days, approximately 20 minutes per day

Materials and Tools

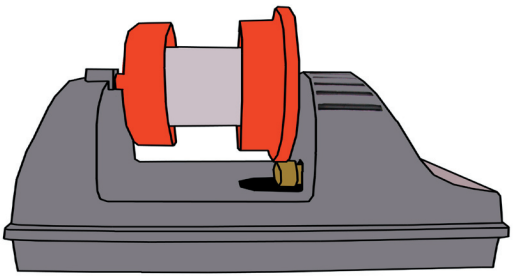
Supplies per group of 3-5 students

1 Classroom Model Bioreactor
3 – 125 mL specimen jars
  • 1 with 5 – ¼" holes in the bottom for drainage
30 – 36 seeds (recommended: Serrano pepper, basil, spearmint)
1 cup of soil
1 toothpick
500 mL of water (bottled or tap)
Paper or cloth towels

Activity Instructions

Growing seeds is not the same as cell culture but it is a good model to take advantage of the mechanism used in culturing cells in the rotating wall vessel bioreactor. In this experiment, students may choose their own types of seeds to grow in several different ways. Groups of 3-5 should have a Classroom Model Bioreactor (CB) and three (3) specimen jars, one of which should have five (5) holes in the bottom about ¼" (0.6 cm) in diameter.

Each group may choose their own seeds to test but should keep it consistent within their experiment. Recommended seeds include: spearmint, basil, and Serrano pepper. Students may test the CB against a stationary control with water and a stationary control will soil. The same number of seeds should be used in each sample. A recommended range for the number of seeds is 10-12.



Notes:

- If time or supplies are limited, simply testing the CB versus the stationary control with water will suffice.
- Another adaptation is to split the samples up among smaller groups. With groups of 2-3 students, give each group one of the samples to examine: CB, water control, or soil control.
- If a soil experiment is to be prepared, try to choose larger seeds such as basil or Serrano pepper.
- Spearmint seeds are very small and difficult to distinguish in soil.

Extensions

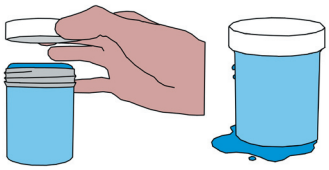
Variations of this experiment include: medium solutes, light exposure, seed types, etc.
With more adaptation, rotation speed can be altered as well as the vessel size.

Sample preparation

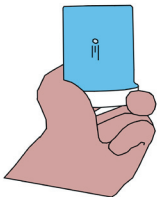
Classroom Model Bioreactor

1. Place 10-12 seeds in a clean, 125 mL specimen jar.
2. Fill the jar half way with water (bottled or tap).
3. Screw the cap on the jar and shake it. This helps the seeds to break the surface tension and sink.
4. Take off the cap and place the jar on a stable, level surface.
5. Using a beaker or cup, pour water to the top of the jar.
6. Very carefully and slowly, continue to pour water to form a meniscus above the level of the jar.

7. Make sure that no seeds are at the top of the jar.
8. Slowly lower the cap, keeping it level, onto the jar and screw it on tightly. Water will spill out but this will prevent air from entering the jar.
9. Turn the jar upside down to check for air bubbles. If you see bubbles, repeat steps 6-9.



10. If there are no bubbles, place the specimen jar in the CB tumbler with the gaskets as shown in the diagram.



11. Place the CB near a window and plug in.

Stationary control with water

1. Follow Classroom Model Bioreactor steps 1-9 using the same number of seeds and the same water source.
2. Tape opaque construction paper (black or another dark color) around the top and bottom. These rings of paper are meant to block out light as do the CB gaskets and tumbler bottom and lid.
3. Place the sample near the window and on its side.

Stationary control with soil

1. Take a specimen jar cap and place it top side down on a level surface.
2. Take the specimen jar with five (5) holes in its bottom and place it right side up in the cap (the cap will act as an overflow tray).
3. Fill the jar ¾ full with soil.
4. Pack the soil down lightly to approximately ½ full.
5. If the soil is not moist, evenly pour about 20-30 mL of water over the soil.
6. Using a toothpick or a piece of wire, loosen and clear a trough in the soil around the sides of the container to the “planting depth” recommended in the seed packet directions.
7. Evenly disperse the seeds in the trough (seeds should be touching the jar’s sides making them visible beneath the soil).
8. Use your finger to lightly fill in the trough with soil over the seeds.
9. Check the moistness of the soil daily and water if needed.

Data Collection

Classroom Model Bioreactor
On a daily basis, at the same time each day:

1. Remove the specimen jar from the tumbler.
2. Count the number of germinated seeds.
3. Measure the length of each germinated seed in millimeters.
4. Record the range of stem/root length and the average length.
5. Replace the specimen jar in the tumbler.

For greater accuracy.
1. Remove the specimen jar from the tumbler. Remove the seeds from the jar.
2. Lightly dab the seeds with paper towels to dry their exterior.
3. Measure the length of germinated seeds in millimeters.
4. Record the range of stem/root length and the average length.
5. Find the mass of all the seeds.
6. Find the mass of the germinated seeds.
7. Record the average mass of each group.
8. Replace the seeds in the jar.
9. Repeat steps 6-9 of the CB Preparation instructions.
10. Replace the specimen jar in the tumbler.

Stationary control with water
On a daily basis, at the same time each day:

1. Count the number of germinated seeds.
2. Measure the length of each germinated seed in millimeters.
3. Record the range of stem/root length and the average length.
4. Replace the jar on its side in the window.

For greater accuracy:
1. Remove the seeds from the jar.
2. Lightly dab the seeds with paper towels to dry their exterior.
3. Measure the length of germinated seeds in millimeters.
4. Record the range of stem/root length and the average length.
5. Find the mass of all the seeds.
6. Find the mass of the germinated seeds.
7. Record the average mass of each group.
8. Replace the seeds in the jar.
9. Repeat steps 6-9 of the CB Preparation instructions.
10. Replace the jar on its side in the window.

Stationary control with soil
On a daily basis, at the same time each day:

1. Count the number of germinated seeds (this may be difficult depending upon how close the seeds are to the side of the jar).
2. Measure the length of each germinated seed in millimeters.
3. Record the range of stem/root length and the average length.
4. Replace the jar in the window (an additional variable is the side of the jar facing the window).

For greater accuracy:
NOTE: Removing the seeds from the soil may damage the roots and stems due to the resistance of the soil. If you choose to remove the seeds:
1. Loosen the soil around each root with a toothpick or a piece of wire.
2. Rinse the seeds.
3. Lightly dab the seeds with paper towels to dry their exterior.
4. Measure the length of germinated seeds in millimeters.
5. Record the range of stem/root length and the average length.
6. Find the mass of all the seeds.
7. Find the mass of the germinated seeds.
8. Record the average mass of each group.
9. Carefully, replace the seeds in the soil along the sides of the jar.

Classroom Bioreactor:
Seeds in Motion

Name(s) _____
_____
_____
_____
_____

What type of seed did you choose for your experiment? _____

How many seeds are you using in each sample? _____

Draw a picture of one of your seeds in the box to the right.

Make a prediction about the growth between the seeds in each environment. Predict regarding the speed of germination, percent of germination, amount of growth, and/or shape of plants.

_____
_____
_____
_____

Data Collection
For each day, fill out the following tables with the required information.

How many seeds in each sample have germinated and what fraction of the total in each sample have germinated? Example: 3 seeds germinated out of 12 → 3/12 = ¼

Day	CB # of seeds	CB fraction	Water # of seeds	Water fraction	Soil # of seeds	Soil fraction
1						
2						
3						
4						
5						
6						
7						
8						
9						
10						

Data Collection, *continued…*

How long are the **shortest sprouts** and **longest sprouts** in each sample?

Day	CB shortest	CB longest	Water shortest	Water longest	Soil shortest	Soil longest
1						
2						
3						
4						
5						
6						
7						
8						
9						
10						

What is the **average length** and **average mass** of the sprouts in each sample?

Day	CB avg. length	CB avg. mass	Water avg. length	Water avg. mass	Soil avg. length	Soil avg. mass
1						
2						
3						
4						
5						
6						
7						
8						
9						
10						

Data Analysis

Which sample had the shortest germination time? _____

Which sample grew the longest sprouts? _____

Which sample had the largest average length? _____

Which sample had the largest average mass? _____

Summary

Was your prediction correct? Why do you think so?

_____

_____

What could you change in the experiment to test further?

_____

_____

Glossary

aggregate: a mass, assemblage or sum of cells
antibodies: protein molecules produced as a primary immune defense
aseptic: free from septic matter or disease producing bacteria
cancer: a malignant new growth anywhere in the body of a person or animal
carcinoma: any malignant tumor derived from epithelial tissue
cartilage: a tough, elastic, fibrous connective tissue found in various parts of the body, such as the joints, outer ear, and larynx; a major constituent of the embryonic and young vertebrate skeleton, it is converted largely to bone with maturation
coculture: growth of different cell types in the same culture to study their interaction
differentiation: the process by which cells or tissues undergo a change toward a more specialized form or function
disease: to interrupt or impair any or all the natural and regular functions of an organ in a living body
expression: the physical manifestation of a gene
genes: any of the elements by which hereditary characteristics are transmitted and determined
germination: to sprout or bud
glycosaminoglycan: Any of a group of linked sugars with high molecular weight that contain amino sugars and often form complexes with proteins
gravity: The natural force of attraction exerted by a large body, such as Earth, upon objects at or near its surface directed toward the center of the body.
***in vitro*:** an artificial environment outside of a living organism
***in vivo*:** inside a living organism
incubator: an apparatus in which environmental conditions, such as temperature and humidity, can be controlled
infectious: caused by a pathogenic agent
International Space Station (ISS): an experimental laboratory established using International collaboration orbiting around Earth
malignant: cancerous
media: the substance in which a specific organism lives and thrives
metastasis: transmission of cancerous cells from an original site to other sites
microenvironment: the immediate physical and chemical surroundings of a microorganism
musculoskeletal: relating to or involving the muscles and the skeleton
mutate: change in genetic material inside a cell
orientation: position and bearing relative to a fixed point
parasite: an organism that grows, feeds, and is sheltered on or in a different organism while contributing nothing to the survival of its host
propagation: an increase or growth
protein: any of a large group of nitrogenous organic compounds that are essential constituents of living cells
renal: relating to or in the region of the kidneys
shear force: a tangential force acting on one face of an object while the opposite face is held fixed
Space Transportation System (STS): part of the NASA Space Shuttle mission number. All space shuttle missions are numbered with this prefix.
terminal velocity: the fastest an object can fall through a fluid due to resistance from the fluid
terrestrial: existing on Earth
three-dimensional: having a specified number of dimensions, measurable as a cube as a three dimensional object
tissue culture: the process or science of growing tissue artificially in a special medium
transplant: to transfer tissue or an organ from one part of the body or from one individual to another

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Unsworth BR and Lelkis PI. Growing Tissues in Microgravity. Nature Medicine 4: 901-907, 1998.

Evaluation:

The activities in this educational brief help students achieve mastery of national standards for mathematics, science and technology, including:

Principles of Standards for School Mathematics by National Council of Teachers of Mathematics, 2000, Grades 9-12, Understand measurable attributes of objects and the units, systems, and processes of measurement.

National Science Education Standards by the National Research Council, 1996, Grades 9-12 explaining concepts, processes, characteristics and change in biological properties as a result of change in gravity conditions.

Standards for Technological Literacy: Content for the Study of Technology by the International Technology Education Association, 2000, Grades 9-12 explaining advances and motivations in medical technologies.

Web sites:

http://SpaceResearch.nasa.gov
http://microgravity.nasa.gov
http://science.nasa.gov
http://kids.msfc.nasa.gov
http://asgsb.indstate.edu/factsheets/plant.html
http://slsd.jsc.nasa.gov/bso
http://nasaexplores.com

Please take a moment to evaluate this product at
http://www.ehb2.gsfc.nasa.gov/edcats/educational_brief
Your evaluation and suggestions are vital to continually improving NASA educational materials.
Thank You.